A STUDY OF THE THRUSH PARASITE

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The organism of thrush was discovered by Berg [1] in 1839. Robin, quoted by Plaut,[2] in 1847 named the organism Oidium albicans, a name it has held most constantly up to the present day. The discovery led immediately to botanical studies of the organism. Many works have been published, but unfortunately these are lacking in agreement. A glance into bacteriological textbooks reveals but meager descriptions of this organism. Points on which reports vary most widely are its morphology and botanical position and the unity or plurality of species.

Microscopically the thrush organism may appear in yeast-like or filamentous form. The filaments are simple or branched, definitely septate, showing thick cross walls. The cells contain protoplasm, vacuoles, granules, a nucleus and fat globules. The contents in young cultures are homogeneous, in older cultures vacuolated. Simple filaments may give rise to short globular buds at their sides. These buds may in turn elongate and form branches, and these branches may again bud and again branch out. This often leads to such an interlacing of filaments that it is impossible to follow any one.

The formation of filaments, according to Linossier and Roux,[3] may take place by two distinct processes. Sometimes a bud appears on the cell which separates off immediately by a manifest septum. It does not remain round but lengthens, becoming the segment of a hypha, and reproduces other segments by a similar mechanism. Or the initial yeast cell may push out a prolongation like a finger of a glove, which is not separated by a septum and retains the same protoplasm.

The formation of filaments, according to Linossier and Roux,[3] may They bud actively. As a rule the buds separate on attaining mature size and form new daughter cells, but sometimes they remain attached, presenting the appearance of beads or bouquets.

Received for publication Aug. 10, 1920.

[1] Ueber die Schwämmchen bei Kindern, 1842.

[2] Kolle und Wassermann Handbuch der path. Mikroorg., 1913, 5, p. 42. Deutsch. med. Wchnschr., 1894, 20, p. 920.

[3] Compt. rend. de l'acad. d. sc., 1889, 109, p. 752. Arch. de med. exp. et d'anat. path., 1890, 2, p. 62.

In the false membranes, or "plaques," found in the mouth, the yeast-like cells are attached to the filaments, or free. The attachments are lateral or terminal and so resemble conidia that many authors use the term "conidia" in speaking of the globular forms.

In cultures the organism appears either in the pure yeast form, or in the pure mycelial form (though this is rare) and frequently in both forms. The appearance of either or both of these forms depends on the composition of the medium and other factors which will be discussed later.

The first obstacle which presents itself in the study of the parasite is that of classification and systematic position. Since its discovery the organism has been named as follows: Sporotrichum by Gruby, quoted by Plaut, Stemphylium polymorphum by Hallier,[4] Mycoderma vini by Grawitz,[5] Dematium albicans by Laurent,[6] Mucor by Linossier and Roux,[3] Syringospora by Quinquaud,[2] quoted by Plaut, Saccharomyces by Guidi, Reess, quoted by Plaut,[2] Fisher and Brebeck,[7] and Audry,[8] Endomyces albicans by Vuillemin,[9] and Oidium lactis albicans by Robin, quoted by Plaut.[2]

But, as Plaut [2] points out, Gruby named it Sporotrichum because of the side ectospores; but many fungi form such ectospores. Stemphylium has brown or black spores and in no way resembles the thrush parasite. Mycoderma vini is out of the question because it is non-pathogenic. The thrush parasite differs from Dematium in the manner of spore formation. The mere finding of chlamydospores in this organism is not sufficient to name it Mucor, for chlamydospores are common to many fungi. The distinct septation of the thrush filaments separates it from Phycomycetes. Its action toward chemicals, its pleomorphic forms, and the presence of chlamydospores removes it from Saccharomycetes.

The problem narrows down to the question of classifying the organism in the genus Endomyces or the genus Oidium.

According to Stevens,[10] the genus Endomyces belongs to the family Endomycetaceae, order Saccharomycetales. The characteristic features of the family are a mycelium usually well developed, producing

[4] Botan. Ztg., 1865, 23, p. 253.
[5] Virch. Arch., 1886, 70, p. 546.
[6] Bull. Soc. belge de micr., 1890, 6, p. 14.
[7] Zur Morphologie, Biologie und Systematik der Kahmpilize, der Monilia Candida und des Soorerregers, 1894.
[8] Rev. de med., 1887, 7, p. 586.
[9] Revue mycologigue, 1899, 21.
[10] Fungi Which Cause Plant Disease, 1913.

a luxuriant growth, multiseptate; asci borne singly on branches or intercalary, 4-8 spored, and unicellular conidia produced apically. The genus Endomyces is characterized by asci, 4 spored. The genus Oidium belongs to the family Oösporeae, order Moniliales, of Fungi imperfecti, since it produces no ascospores.

If the presence of asci and ascospores as mentioned by Fisher and Brebeck can be verified, we are justified in classifying the thrush parasite with Endomyces. The absence of these structures leaves for the organism no other genus but that of Oidium.

Three types of spores have been described as occurring in the thrush parasite, namely, conidia, chlamydospores, and ascospores.

The yeast-like globules or conidia forms are surrounded by a membrane slightly thinner than that of a pure yeast. This membrane gives no cellulose reaction. Within the membrane are cytoplasm and nucleus. The cytoplasm contains granules and vacuoles. Within the vacuoles can be seen the little dancing granules which are so characteristic of yeast cells. In young cultures the protoplasm is hyaline and homogeneous. In older ones it becomes vacuolated and granular. It takes the basic aniline dyes and retains Gram's stain. The nucleus is small but can be distinctly seen with Heidenhain's iron hematoxylin. It is surprising to read Linossier's and Roux's [3] statements that they could detect no nucleus.

According to Linossier and Roux,[3] these globular cells are not spores but the vegetative phase of the plant, which can adapt themselves to all sorts of mediums. Vuillemin [9] believes them to have the functions of both spores and vegetative cells. Daïreuva [11] states that the "conidia" bud when conditions are favorable; when unfavorable these are able to resist drying, abnormal temperature and lack of food by changing into mycelium.

Chlamydospores are described by Linossier and Roux [3] as follows:

> The form is characterized by certain filaments at the extremities of which are spherical cells. These cells or chlamydospores are larger than the conida, sizes ranging from 9-24 mikrons. They may also be round between two segments. They are spherical and their protoplasm is more refringent and granular than that of the "conidia" or filaments. Their membrane is thicker. The protoplasm in the chlamydospores is at first finely granular and but slightly refractile; later it becomes coarsely granular. The granules are either arranged like a necklace or bunched, surrounding a central hyaline globule. The surrounding membrane of the terminal cell thickens and become glassy. On squeezing, it always opens at the same point by a V-shaped rent through which the granules with the central globule escape. Preceding this process glycogen accumulates in the preterminal cells, as shown by staining with iodine. These globules, as they escape, remain indefinitely in the media without further change, but have been made to germinate on raw cherries. They are believed to be chlamydospores which germinate in order that the parasite may develop in a new habitat.

The medium in which, according to these authors, the chlamydospores appear constantly is a liquid, each liter of which contains saccharose,, 20 gm.; ammonium tartrate, 10 gm.; potassium phosphate, 1 gm.; magnesium sulphate, 2/10 gm., and calcium chloride, 1/10 gm. This medium was used for many of my studies.

Plaut[2] considered chlamydospores as involution forms of thrush. Burchardt (quoted by Plaut[2]) described "capsules" which he found in emulsified false membrane. He described them as being round, 1/50-1/12 mm. in diameter, double contoured and full of small spores. He considered them to be sporangia. Hausman (quoted by Plaut[2]), and Hallier[4] also mentioned capsules which they called sporangia. Grasset (quoted by Plaut[2]), found chlamydospores in old broth cultures. Charrin and Ostrowsky[12] found some in dextrose broth cultures. Hickel[13] described them as round cells three times the size of the ordinary cells, full of reserve food and surrounded with a thick refractile membrane. Vuillemin[9] and Daireuva[11] have found them in old cultures and believe that they arise when conditions are unfavorable, such as lack of nutrition, presence of bacteria, or chemical influences. They observed their germination on transplanting them from beets to broth. I found them in Linossier's medium in two of my strains.

Vuillemin[9] and Fischer and Brebeck[7] have described ascospores. Vuillemin states that they are numerous in old cultures on various mediums and that asci 4-5 mikrons in diameter contain 4 ascospores.

Fischer and Brebeck found them in 5 cases of thrush. The strains formed a pellicle on milk; 14-day old cultures of these showed endospores. They also isolated a nonpellicle forming kind from a sixth case of thrush, but in this case they could find no endospores.

In infected tissue both "conidia," or spherical cells, and mycelium can be seen. In artificial medium we find the globular form alone under certain conditions, and obtain the mycelial or filamentous form under other conditions. All of the authors except Stumpf[14] agree on the existence of a globular, as well as a filamentous form, but they do not all agree as to where and why each form appears.

Grawitz[5] used a liquid medium made by adding to dextrose solutions ammonium tartrate and 2% of a mineral salt obtained from ashes. He used various concentrations of sugar and claimed that the greater the concentration of sugar in the medium the more the organism takes on the yeast form. Plaut[2] stated that he obtained mycelium in sugar-free nitrogenous medium, and yeasts in rich sugar medium. Audry[8] believed that solid mediums cause the growth of the yeast form while liquid mediums cause the growth of the mycelial form. He planted the organism on lemons and obtained the pure globular forms, some free and some attached like pearls on a string. In broth, he said the round cells became oval, elongated and attached. Some gave rise to long filaments which bulged at one end. This bulging part pediculated in some cases and formed small adherent cells. The filaments were septate and rounded at their extremities.

Nearly all the authors are in agreement that yeast-like cells alone appear on the surface of solid mediums while mycelium may develop in some liquid mediums. The most elaborate research on this aspect has been made by

[11] Recherches sur le champignon du muguet et son pouvoir pathogene, 1899.

[12] Comptes rend. Soc. de biol., 1896, 48, p. 743.
115, p. 159.

[13] Sitzungsber. d. math.-naturwiss. Klasse der Akad. d. Wissensch. in Wien, 1907, Pt. 1.

[14] Aerztl. Intelligenzbl., 1885, 32, p. 627.

Linossier and Roux,[8] who after a thorough study of the organism on a variety of mediums arrived at the theory that "the complexity of form of the thrush organism is proportional to the increase in the molecular weight of the food elements in the medium." As a proof of their theory they cite the following experiment:

They planted the organism in a mineral liquid consisting of water 1,000 c c, potassium phosphate 0.75 gm., magnesium sulphate 0.05 gm., ferrous sulphate 0.02 gm., zinc sulphate 0.02 gm., a trace of sodium silicate and ammonium sulphate 1 gm., and to this were added carbohydrates of various molecular weights. In the mediums of low molecular weight, such as lactose, glucose or glycerol, they obtained only yeast forms. In mediums of high molecular weight, such as dextrin or gum arabic, they obtained long abundant filaments.

They do, however, admit that this rule is not without exceptions; for they obtained filaments in acid, alkaline and nitrogenous mediums of low food value, and under conditions which were unfavorable, such as lack of food. They explained the presence of yeast-like cells on the surface of their solid mediums, especially those of high molecular weight, by saying that cells separated from the medium by other cells receive that medium by diffusion; only simple foods are diffusible, and therefore the organisms grow in the simple or yeast-like form. Cells directly in contact with the medium, namely, in a liquid medium, develop at its expense and consequently grow in mycelial form.

Hickel[13] accepted Linossier and Roux's theory as "to the complexity of form in proportion to increase of food value," and carried out a similar experiment. He used a medium containing 500 gm. of water, 0.25 gm. of magnesium sulphate, 0.25 gm. of potassium phosphate, a trace of iron sulphate, and 0.25 gm. of ammonium sulphate. To some of this medium he added monoses, such as glucose, levulose, fructose and galactose. To some of it he added bioses, such as maltose, lactose and saccharose; and to some he added two polyoses, namely, dextrin and glycogen. In these he planted the thrush organisms. He found mycelium in the bioses and polyoses, but obtained no mycelium in the monoses.

He also believed that aerotropism brings about the mycelial forms. He said that all his stab cultures showed a characteristic growth at the top of the tube; where the oxygen tension was high he obtained only the yeast form; where the tension was low, namely, toward the bottom of the tube, he obtained the mycelial form.

He further illustrated this theory by the following experiment: He dissolved a tiny piece of sugar in several c c of saliva. To this he added a small amount of the yeast-like cells from a fresh malt culture. He then took a drop of this emulsion and placed it on a slide. The slide was covered with a cover glass and placed in a dish surrounded with wet filter paper so as to provide sufficient moisture. This was then placed in the incubator. At the end of twelve hours the preparation showed mycelial forms toward the center of the drop, for here the oxygen tension was low, and yeast-like forms at the edges.

Concerning the unity or plurality of species there is considerable difference of opinion. Stumpf[14] declared that the filamentous and the globular forms were two different species of fungi. However, no other observer has agreed with this view. Fischer and Brebeck[6] claimed that there were two varieties: one, a large-spored variety that liquefied beer wort gelatin and formed a pellicle on milk and wort, and formed endospores; the other, a small-spore

kind that does not liquefy gelatin nor does it form pellicles or endospores. Daireuva[11] has come to the conclusion that "neither the microscopic, pathologic or cultural aspects warrant the establishment of specific differences."

Sugars have been found useful in classifying bacteria, such as the coli typhoid group. No attempt that we know of has been made to classify the thrush organisms by this means. The authors who have mentioned sugars are Cao,[15] Fisher and Brebeck,[7] Daïreuva,[11] Troisiér and Achalme,[16] and Denecke, quoted by Plaut.[2] Cao, in describing the thrush organism, best known in literature as Oidium albicans, stated that it attacked no sugars. Fischer and Brebeck said that the liquefying variety fermented dextrose, levulose and maltose but not saccharose. Daïreuva stated that the parasite fermented glucose, levulose and maltose; that it consumed dextrin, mannite and glycerol without fermenting; that it did not utilize or ferment lactose, and utilized saccharose without inverting or fermenting. Denecke claimed that it fermented levulose, maltose, lactose but not saccharose. Troisiér and Achalme described a case clinically diagnosed as thrush. They did not, however, believe the organism isolated from the case to be the thrush parasite, but rather a yeast. One of the points on which they based this decision was the fact that their organism fermented saccharose and showed strong alcoholic fermentation. They believed that the thrush parasite did not do this.

Agglutination is another means in use in modern bacteriology for establishing species. Noisette, quoted by Plaut,[2] has carried out some agglutination tests with his thrush cultures. He says that on immunizing an animal with a thrush strain, the serum of that animal develops agglutinins, which agglutinate the specific strain that has been used for immunizing. He has tried this serum on various strains but has found that the serums will agglutinate only their own specific antigens. He has, therefore, concluded that there is not merely a single Saccharomyces albicans, but an entire class, which contains varieties.

Roger[17] succeeded in obtaining agglutinins in immunized rabbits. More recently Widal and others,[18] in studying agglutination with the serum of sporotrichosis cases, have discovered that the serum of patients who suffered from thrush will agglutinate the "conidia" of Oidium albicans, but only in low dilutions, 1:10 to 1:50, and that the same serum agglutinates more markedly Sporotrichum spores in dilutions of 1:40 to 1:50. This reaction with the spores of Sporotrichum beurmanni is so constant that they were able to use it in the diagnosis of thrush. With the exception of Noisette, no author has tried to establish the unity or plurality of species by means of agglutination.

I began the study of the thrush parasite with 2 strains on hand, T 2 and T 9; the first from the Army Medical School and the other from the university clinic, both from clinically diagnosed thrush cases. Within 6 months there were added 15 more strains; T 26 and T 27, from vaginal cases in pregnant women; T 13, from a case of conjunctivitis; T 11, T 12 and T M, from typical cases of mouth thrush; T L, from an ulcer in the mouth, and the remainder, T 14, T 16, T 18,

[15] Ztschr. f. Hyg. und Infectionskr., 1900, 34, p. 282.
[16] Arch. de méd. expér. et d'anal. path., 1893, 5, p. 29.
[17] Compt. rend. Soc. de Biol.. 1896, 48, p. 728.
[18] Ann. d. l'Inst. Pasteur, 1910, 24, p. 1.

T 21, T 22, T 23, T 24, T 25, were obtained from throat cultures sent to the laboratory of the Minnesota State Board of Health. The latter were cultures taken for diagnosis of diphtheria. For purposes of comparison yeast strains were used. Of these Y 1 was a cultivated yeast, Y 2, Y 3, Y 4 and Y 5 were wild yeasts and Y G, Y 17 and Y 19 were yeasts isolated from the throat cultures sent to the laboratory of the State board of Health. A monilia, from a case of sprue, obtained from the Army Medical School, was also used.

Pure cultures of these strains were obtained by plating them on dextrose-tartaric acid agar. This medium is made by adding to 10 c c of melted agar 1 c c of a sterile dextrose tartaric acid solution (prepared by adding 50% of dextrose and 5% of tartaric acid to water, sterilized in the autoclave). The addition of the dextrose tartaric solution inhibits the growth of bacteria and makes easy the obtaining of pure cultures. A good growth is obtained within 2 days.

The surface colonies are round, wax-like, creamy, elevated and granular; while the deep colonies are irregularly surrounded with radiating mycelium. Some of these deep colonies are round and are surrounded with a fine branching mycelium so that they present a stellate appearance; others are torpedo shaped with the mycelium extending from one side.

On routine mediums my strains of the thrush organism gave no characteristic growth. They grew at the bottom of broth in flocculent form. On milk there was no change, nor did any pellicle form. On gelatin there was a smooth white growth on top. None of my strains liquefied maltose gelatin. On agar, the growth was fine and whitish; on potato, it was gray. The organism grows most easily and abundantly on Sabouraud agar. I also planted my strains, as well as some yeasts, on carrots. Both gave a snow white growth. This, therefore, cannot be used for the differentiation of the thrush organism as Linossier and Roux [3] claim. The morphology of the organism has been described in the earlier part of this paper. As endospores were not observed in the numerous strains and wet preparations which I made from various mediums, I tried the gypsum block method, hoping to obtain them that way. Plaster of Paris was hardened, slanted, fashioned to fit into glass tubes, moistened with peptone solution or distilled water and autoclaved. The various strains of the parasite were inoculated on the slanted surface of the gypsum. No nutritive material was placed in the tubes, for I wanted to create unfavorable conditions which would result in sporulation, just as is the case with

yeasts. The tubes were kept at a temperature of 20 degrees for several days.

At the end of that time it could be seen that the cells became oval, larger and swollen. They became free from granules, the dancing figures were enlarged, and the vacuoles distended so that they looked like spores. Carefully made spore stains, using Moeller's method, showed none. The vacuoles appear so much like spores that one can easily be misled into believeing them to be such.

An attempt was also made to verify the observation of chlamydospores so carefully and elaborately described by Linossier and Roux, using their medium. In this medium, tubed and sterilized, I planted my strains. I found chlamydospores in 2 of them, namely T L and T 2. Their appearance corresponds to the description given by Linossier and Roux. They were spherical and enclosed by a heavy membrane. Within could be seen the highly refractile globule surrounded by a corona of tiny granules. The chlamydospores were at the extremities of short hyphae. I was unable to squeeze out the globules or observe the germination of the chlamydospores.

The complete absence of ascospores or, in fact, of any sort of endospores in all of the strains studied, no matter what the medium, leads me to believe that such structures are not formed by the thrush parasite and that it should, therefore, be retained in the genus Oidium rather than the genus Endomyces.

Chlamydospores are not typical for any genus. They are common to many fungi, hence the finding of them in two of the strains does not affect the classification. I believe them to be a resistant form of spore arising when conditions become unfavorable.

In my cultures I found, as a rule, yeast-like cells on the surface of the solid mediums. However, there were exceptions. Some of the old agar slant cultures developed deep radiating mycelium. Certain of my dextrin and dextrose agar slants, on being subjected to anaerobiosis (described later), showed mycelium, as did also deep colonies in dextrose-tartaric acid agar.

Liquid mediums, as a rule, showed the mycelial form. Here again were exceptions. I have found only yeasts in Linossier's dextrose liquid medium and in another medium devised by Koser and Rettger [19] and containing: water, 1,000 c c; sodium chloride, 4 gm.; KCl, 1 gm.; $MgSO_4$, 0.2 gm.; $CaCl_2$, 0.05 gm.; KH_2PO_4, 1 gm.; $(NH_4)_2PO_4$ 1 gm., and glycerol, 30 gm.

[19] Jour. Infect. Dis., 1919, 24, p. 301.

The addition of carbohydrates to liquid mediums showed marked results. Linossier's medium was used, to which were added dextrose, galactose, or dextrin. These were made up in liquid form, or solidified with agar. The solid mediums gave only the yeast-like cells. Of the liquid mediums, dextrin and galactose gave profuse mycelium and dextrose gave only the globular form. Galactose is of about the same molecular weight as dextrose and, therefore, in accordance with Linossier's theory, should give the yeast cells only. I have also obtained mycelium in sugar-free medium, such as plain broth and peptone. These results seem to show that sugars have some influence on the formation of mycelium, but not to the extent to which Linossier and Roux would have us believe.

Two other experiments which I have carried out seem to indicate that other factors, besides the composition of the medium, influence the morphology of the organism. These experiments were carried out to observe the influence of oxygen and the surface tension of the medium.

Linossier's mineral liquid medium was made up with dextrose, dextrin and galactose, and is dextrin and dextrose agar slants. On these were planted 5 strains, namely, T 9, T 12, T 26, T M and T 16. The tubes were immediately placed in jars which were connected by means of glass tubing to a flask containing calcium carbonate. Just before adding hydrochloric acid to the calcium carbonate for the purpose of generating carbon dioxide, there was added to each jar some pyrogallic acid and sodium hydroxide solution. Carbon dioxide was then passed through the jars. When a match would no longer burn in the gas escaping from the last jar in the series, the apparatus was disconnected and the glass tubing quickly sealed. The jars, together with some controls grown aerobically, were placed in the incubator. After two days the cultures were examined. Table C shows the results. It shows that anaerobiosis brought about a mycelial growth on the solid medium of most of the strains planted, but there are some exceptions.

The work of Larson, Cantwell and Hartzell [20] on the effect of surface tension of the medium on the growth of bacteria has given interesting results. It was thought that the surface tension of the medium might have some influence on the growth of the thrush parasite, so the following experiment was made. Linossier's liquid medium

[20] Jour. Infect. Dis., 1919, 25, p. 41.

was again used. The surface tension was depressed by the addition of castor oil soap prepared as described by the authors. The dextrin solution without the soap had a surface tension of 50 dynes. The addition of 1% of a 2% solution of the castor oil soap depressed the tension to 44 dynes. The galactose medium had a tension of 48 dynes and 43 when depressed as above. Dextrose changed from 58 to 43 dynes. Seven strains, T 9, T 12, T 16, T 26, T M, T 2, and T L were

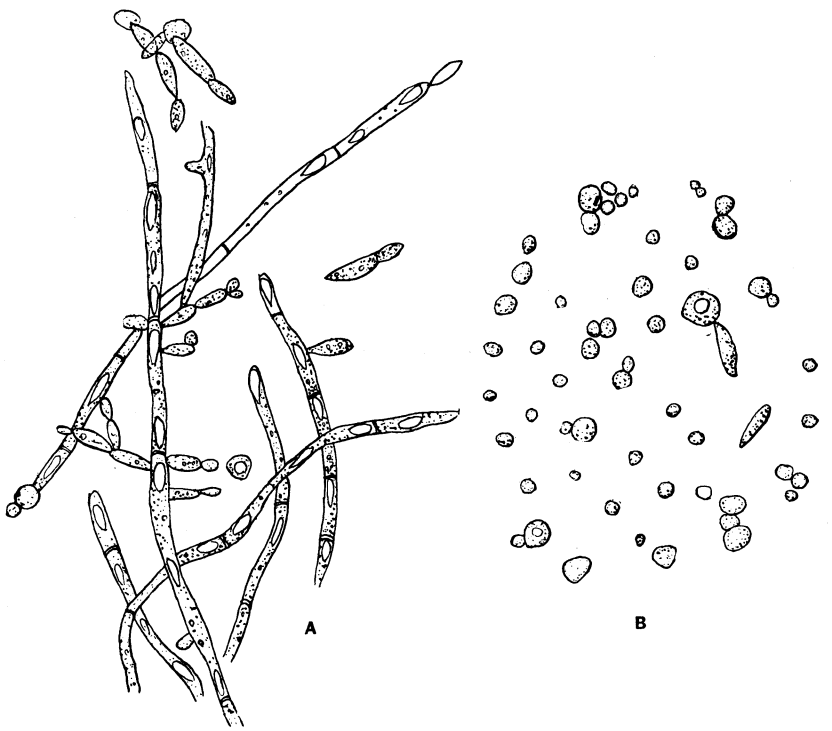

Fig. 1.—A, the morphology of Oidium albicans in Linossier's solution with dextrin, normal surface tension; B, the same strain grown in Linossier's solution with dextrin, the surface tension being depressed by castor oil soap.

inoculated simultaneously into tubes of these mediums, both of normal tension and of low tension as described, and incubated at 37 degrees. Table C shows the results after two days' growth. It shows that in the case of dextrin the filamentous form was present in controls, while yeast-like forms alone appeared when the tension was depressed. The results were most striking and definite. They are presented in figure 1, A.

With galactose, however, no such differentiation was observed, mycelium appearing in fluid of low tension as well as in the normal control tubes, while with dextrose also the addition of soap was without influence, the yeast form alone appearing in both series of tubes. After making these microscopic observations, the tubes of each of the inoculated mediums, both those of the normal tension and those of low tension, were centrifuged and the surface tension of the clear supernatant fluid was read. The surface tensions were determined by Mr. Green using Du Nouy's apparatus. The results are shown in the following table:

TABLE 1

SURFACE TENSION OF CLEAR SUPERNATANT FLUID AFTER CENTRIFUGATION OF MEDIUMS

	Uninoculated Normal Medium	Uninoculated Depressed Medium	Inoculated Normal Medium	Inoculated Depressed Medium
Dextrin	50 dynes	44 dynes	54.5 dynes	48 dynes
Galactose	48 dynes	43 dynes	65 dynes	49.5 dynes
Dextrose	53 dynes	43 dynes	58 dynes	46.5 dynes

It will be seen that the growth of the organism in every case raised the surface tension of the medium. This increase, however, is much more marked in the case of galactose and dextrose than dextrin, and is probably to be explained by the acid produced from the simpler sugars, which will precipitate soap.

Unfortunately these studies could not be carried further. It is quite clear, however, from the work done, that a multiplicity of factors determine the form which the thrush parasite may assume. In general, I may state that the yeast or unicellular form occurs in the optimum mediums. The organism grows most rapidly when the medium contains an abundance of the simpler fermentable carbohydrate. It is aerophilic and produces a more luxuriant growth on the surface of solid medium than in the depths of liquid medium. The unicellular form, the cells being spherical or oval, offers a smaller surface in proportion to the volume of the protoplasm than the cylindrical mycelium, but also affords the most rapid means of reproduction and dissemination of the organism.

It would seem that the yeast-like form is, therefore, the optimum form and that the mycelial form is only assumed in mediums poor in

TABLE 2

SUGAR FERMENTATIONS, FIRST SERIES

Strain	Arabinose		Amygdalin		Dextrose		Dulcit		Erythrite		Galactose		Glycerin		Inulin		Lactose		Levulose		Maltose		Mannite		Raffinose		Saccharose		Starch		Salicin		Xylose	
	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Y1	+	—	+	—	+	+	—	—	—	—	+	+	+	—	+	—	+	—	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	—
Y2	+	—	—	—	+	+	—	—	—	—	+	—	+?	—	+	—	—	—	+	+	+	+	+	—	+	—	+	+	+	+	—	—	—	—
Y3	—	—	+	—	+	+	—	—	—	—	+	+	+	—	—	—	—	—	+	+	+	+	+	—	+	+	+	+	+	—	—	—	—	—
Y4	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—	—	—	+	—	—	—
Y5	+?	—	+	—	+	+	—	—	—	—	+	—	—	—	+	—	—	—	+	+	+	—	+	+	+	—	+	+	+	—	—	—	+?	—
T2	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	—	—	—	—	—
T9	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	—	—	—	—	—
T11	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	—	—	—	—	—
TL	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	—	—	—	—	—
TM	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	—	—	—	—	—
Monilia from sprue	—	—	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	+	—	—	—	—	—	—	—

"A" indicates acid, "G" indicates gas, "Y" indicates yeast strains, "T" indicates thrush strains.

oxygen, or in readily assimilable carbohydrates, this form possibly being better adapted by virtue of its larger surface for absorption and respiration.

The task of differentiating species of bacteria has been greatly aided by the use of sugars and agglutination tests. I resorted to these means in an attempt to determine the unity or plurality of species of thrush organisms.

TABLE 3

SUGAR FERMENTATIONS, SECOND SERIES

Strain	Dextrose		Galactose		Lactose		Levulose		Maltose		Mannite		Raffinose		Saccharose		Presence of Mycelium
	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	
YG	+	—	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—
Y17	+	+	+	+	—	—	+	+	+	+	—	—	+	—	+	+	—
Y19	+	—	+	—	—	—	+	—	+?	—	—	—	—	—	—	—	—
T2	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T9	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T11	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T12	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T13	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T14	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T16	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T18	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+?
T21	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T22	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T23	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T24	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T25	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T26	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
T27	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
TL	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
TM	+	+	+	—	—	—	+	+	+	+	—	—	—	—	+	—	+
Monilia from sprue	+	+	+	+	—	—	+	+	+	+	—	—	+	—	+	+	+

"A" indicates acid, "G" indicates gas, "Y" indicates yeast strains, "T" indicates thrush strains.

Sugar broths were made by dissolving 1 gm. of the sugars to each 100 c c of beef extract broth, using Andrade's indicator. The following carbohydrates were used at first: dextrose, maltose, lactose, saccharose, mannite, glycerol, salicin, inulin, galactose, arabinose, levulose, amygdalin, xylose, erythrite, raffinose, dulcite and starch. Into these were planted some strains of the thrush organism and some strains of yeasts as controls. Table 2 shows the results obtained. They seemed encouraging, for whereas the yeasts showed a variety of fermentations, the thrush strains presented uniform results. A fresh lot of medium was made. The list of sugars was limited to dextrose, galactose, levulose, maltose and saccharose since on the other sugars there was no action. Again the thrush strains fermented the

same sugars. Whenever I obtained a new strain of clinically diagnosed thrush I planted it in the sugars and invariably obtained the same results. All of the strains studied fermented the following sugars with the results indicated: dextrose acid and gas, galactose acid without gas, levulose acid and gas, maltose acid and gas, saccharose acid without gas.

On repetition of the experiment with all of the strains after several months' cultivation, the same results were obtained. From this experiment it is clear that the thrush parasite is constant in its sugar reactions and that these reactions do not indicate a plurality of species. All of the yeasts studied gave sugar reactions different from those of the thrush strains, so that the sugar reactions can be utilized for the identification of Oidium albicans. They are the more useful in that typical mycelium is formed readily in many of the sugar broths, most constantly and abundantly in galactose.

TABLE 4

INFLUENCE OF OXYGEN AND SURFACE TENSION ON MORPHOLOGY

	Aerobic					Anaerobic					Liquid					
	Solid		Liquid			Solid		Liquid			Normal Tension			Low Tension		
	Dex-trin	Dex-trose	Dex-trin	Ga-lac-tose	Dex-trose	Dex-trin	Dex-trose	Dex-trin	Ga-lac-tose	Dex-trose	Dex-trin	Ga-lac-tose	Dex-trose	Dex-trin	Ga-lac-tose	Dex-trose
T9	Y	Y	M	M	Y	M	Y	M	M	Y	M	M	Y	Y	M	Y
T12	Y	Y	M	M	Y	M	M	M	M	Y	M	M	Y	Y	M	Y
T26	Y	Y	M	M	Y	M	M	M	M	Y	M	M	Y	Y	M	Y
TM	Y	Y	M	M	Y	Y	M	M	M	Y	M	M	Y	Y	M	Y
T16	Y	Y	M	M	Y	M	M	M	M	Y						
T2	..	..	..	..	..	..	..	..	..	..	M	M	Y	Y	M	Y
T21	..	..	..	..	..	..	..	..	..	..	M	M	Y	Y	M	Y
TL	..	..	..	..	..	..	..	..	..	..	M	M	Y	Y	M	Y

"Y" indicates yeast cells, "M" indicates mycelium.

For my agglutination experiments antigens were made from several strains as follows: 10 c c of a sterile 0.8% salt solution with 0.25% of tricresol was added to each of the Sabouraud agar slants. The growth was gently emulsified. The emulsions were transferred to clean tubes. They were heated for one hour at 56° C. in the water bath. A little of each emulsion was planted on a fresh medium and inspected the next day for growth. If they showed growth, the emulsions were again heated and tested. Of such emulsions, 2 c c were injected intraperitoneally into rabbits 4 times, 3 days apart. One week after the last injection they were bled and the serums collected were

used for microscopic agglutination tests in dilutions of 1:10, 1:20 and 1:50. The emulsions used as antigens were prepared by scraping the growth from Sabouraud agar slants into salt solution. This gave emulsions which slowly sedimented but became uniformly turbid on slight agitation. After making the mixtures they were incubated at 37 C. for 2 hours and then placed in the icebox over night. In the first experiment the following strains were used for immunization: T 2, T 3, T 9, T 11 and T L. The same strains were used as antigens. Serum T L agglutinated the homologous strain and strain T 9, both in a dilution of 1:20. Serum T 9 agglutinated its homologous strain at a dilution of 1:50 and strain T L at 1:10. Otherwise no agglutination occurred.

After one more injection the serum of the rabbit inoculated with T 9 was used for a further experiment using, in addition to the yeast and thrush strains mentioned, 2 emulsions of spores of Sporotrichum schenckii. These were both isolated from cases of cutaneous sporotrichosis. A further test was made using the serums of the 2 cases of vaginal thrush with their homologous strains and with emulsions of sporotrichum spores. The results were completely negative.

It would appear from these experiments that the agglutinins are not formed in sufficient quantity either in experimentally inoculated animals or in clinical cases of thrush to be of diagnostic or differential value. I am unable to confirm with Sporotrichum schenckii the observation of Widal and others [18] with Sporotrichum beurmanni.

SUMMARY AND CONCLUSIONS

Seventeen strains of the thrush parasite proved identical and constant in their morphologic and cultural characters. They all corresponded to the nonliquefying type of Fischer and Brebeck.

Carbohydrate mediums were fermented uniformly and constantly by all strains. They are of value in the identification of the species.

Agglutinins are not produced by the thrush parasite in sufficient quantity to be of diagnostic or differential value.

The thrush parasite produces chlamydospores but not ascospores. It is correctly placed in the genus Oidium.

The organism tends to assume a mycelial form in liquid mediums, in mediums containing complex carbohydrate, in mediums of low oxy-

gen tension, and in mediums of low surface tension, while the unicellular or yeast-like form occurs in solid mediums, in the presence of simple carbohydrates, an abundance of oxygen, or mediums of higher surface tension. These factors may be interrelated, while other factors as yet unknown may affect the morphology. It is suggested that pleomorphism of this organism is an attempt at adaptation, the mycelial form developing in relatively unfavorable conditions.